Mismatch repair: **The praying hands of fidelity**

Josef Jiricny

High-resolution crystal structures have recently been solved for the mismatch binding protein MutS of *Escherichia coli* and its *Thermus aquaticus* homologue; they show how these factors recognise such structurally diverse substrates as base–base mismatches and insertion/deletion loops.

Address: Institute of Medical Radiobiology of the University of Zürich and the Paul Scherrer Institute, August Forel-Strasse 7, CH-8008 Zürich, Switzerland.
E-mail: jiricny@imr.unizh.ch

Current Biology 2000, 10:R788–R790

0960-9822/00/$ – see front matter

The highly conserved post-replicative mismatch repair system improves the fidelity of DNA replication by up to three orders of magnitude by eliminating from the newly synthesised strand errors introduced by DNA polymerases, such as base–base mismatches and unpaired nucleotides (insertion/deletion loops). In *Escherichia coli*, binding of error site by the mismatch recognition factor MutS triggers the assembly of a 'repairosome' containing MutL, MutH, a DNA helicase, one of several exonucleases, a single-strand DNA-binding protein, the replicative DNA polymerase and DNA ligase [1]. Although the respective roles of these proteins in mismatch repair are largely known, the individual steps are not understood in detail.

Mismatch recognition is one of these poorly understood steps. What are the structural determinants recognised by MutS during the binding of substrates as diverse as a G/T mismatch, which has been reported to exist in the form of a wobble-base pair, or an unpaired nucleotide, which causes bending in the DNA? New insights into this puzzling phenomenon have come from the recently determined crystal structures of two closely related MutS proteins, one from *E. coli* [2] and one from *Thermus aquaticus* [3], in a complex with an oligonucleotide heteroduplex carrying a G/T mispair or a single unpaired thymidine, respectively.

In the co-crystal with its DNA substrate, MutS is present as a homodimer, shaped as an oval disk. It has two openings, of diameters approximately 30 Å and 40 Å, with the DNA passing through the latter. The structure can be better visualised as a pair of praying hands, with the thumbs folded inwards, and the DNA passing between the fingertips and the thumbs as shown in Figure 1. Each subunit consists of five distinct domains. The amino-terminal domain I, which forms the top segment of the thumb, contains the conserved amino-acid motif that is required for mismatch recognition (see below). Domains II and III form the second and third thumb segments; domain IV forms the fingers; and the carboxy-terminal domain V the heels of the palms. This last domain contains the protein's ATP-binding site, which consists of the highly conserved Walker-type motifs.

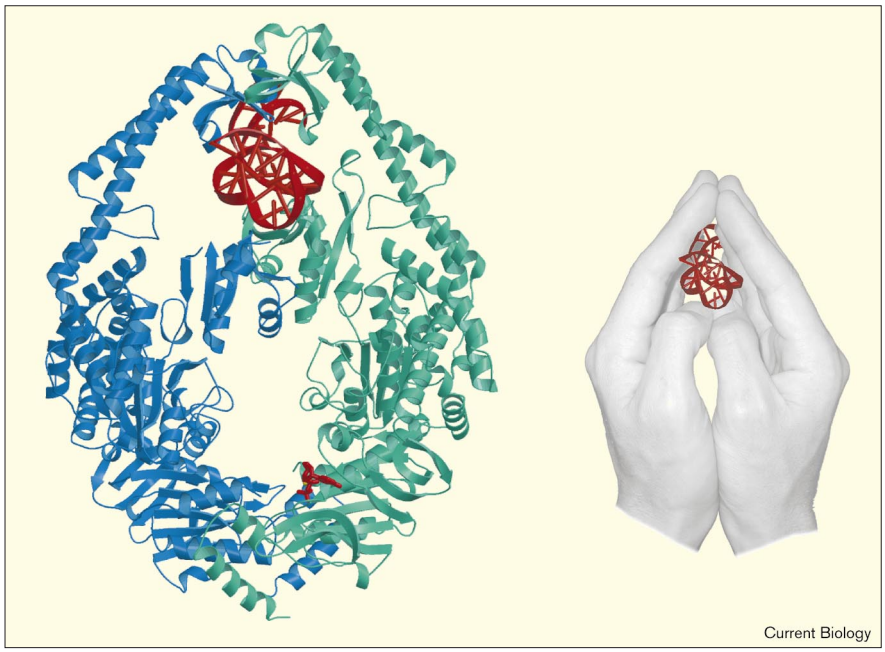
Largely because two α helices of domain V of one subunit help form the ATP-binding pocket of the other subunit, MutS also forms a dimer in the absence of DNA. But unlike the situation in the protein–DNA co-crystal, where the fingers and the top of the thumb can be seen to embrace the DNA, these domains are disordered in the structure of the protein alone [3]. One explanation for this apparent lack of order in the absence of bound DNA is that the MutS fingertips contain several basic residues, which most likely cause electrostatic repulsion which forces the dimer to assume an open, U-shaped conformation. Entry of DNA into the U would lead to the formation of non-specific contacts between the thumb domain and the negatively charged phosphates of the DNA backbone. The fingers can now close around the DNA, because the positive charge of the basic amino acids would be neutralised through further non-specific interactions with the negatively charged sugar-phosphate backbone. These non-specific interactions might force the DNA to bend towards the major groove, widening the minor groove such that the protein can now interact specifically with one of the bases in the mispair.

It is interesting to note that, although the oligonucleotide substrates used in these studies contained either a G/T mismatch [2] or a single unpaired thymidine [3], the DNA was in both cases bent to a similar extent — about 60° — and the minor groove interactions in both structures involved the thymine and the highly conserved amino-terminal motif GX**F**Y(E). This motif is located in domain I, at the tip of the MutS thumb, where the phenylalanine residue (F) was previously shown to be essential for mismatch recognition in the MutS protein of *T. aquaticus* [4].

This interaction is interesting for three reasons. The first is that, although the MutS–DNA complex appears to have a clearly defined two-fold axis of symmetry, only one thumb tip contacts the DNA at the mismatch site. The protein is thus a functional heterodimer. This is noteworthy, because the most abundant eukaryotic mismatch binding factors are also heterodimers, consisting of the MutS homologues MSH2 and MSH6, and recent experiments have shown that only the latter subunit contacts the mismatched substrate [5,6]. Accordingly, the phenylalanine

Figure 1

Structure of the *E. coli* MutS homodimer bound to an oligonucleotide heteroduplex containing a single G/T mispair [2]. The protein is seen as resting flat on the page, while the DNA molecule (brown) should be visualised as passing through the protein orthogonal to the plane of the paper. The thumb tip of the mismatch binding MutS subunit (green) can be seen to contact the DNA, and the same subunit also binds a molecule of ADP (brown) in the heel of the palm, while the thumb of the other subunit (blue) is twisted downwards towards the palm. (Image courtesy of T. Sixma; adapted from [2].)



and glutamate residues of the amino-terminal motif are conserved in MSH6 homologues, but not in MSH2.

Secondly, unlike in the structure of the free oligonucleotide [7], the G/T mispair bound by MutS is not in a wobble-base pair conformation. Rather, the thymine is rotated towards the minor groove, such that the O_4 atom interacts with the hydrogens at the N_2 and N_3 positions of the guanine, while the N_3 and O_2 positions are available in the widened minor groove for interaction with the glutamate residue of the conserved motif GX**F**Y(E) [2]. In the insertion/deletion loop substrate, the unpaired thymine is neither extrahelical, nor is it intercalated in the DNA. Rather, the thymine is displaced towards the widened minor groove, where it is stabilised by an interaction with a guanine on its 5′ side in the same strand, and the glutamate of the thumb tip [3]. In both structures, the conserved phenylalanine residue can be seen to further stabilise the interaction by partially intercalating into the DNA and stacking with the displaced thymine.

Thirdly, the interaction between the MutS protein and the DNA mismatch is asymmetric, in that MutS can be seen to bind to only one base at the error site. But this interaction does not necessarily mark the nucleotide that has to be corrected, as mismatch repair does not take place at the mismatch, but rather commences at a site that denotes the newly synthesised strand — which carries, by definition, the wrong genetic information — and that site can be several hundred nucleotides away from the mismatch [1].

So how do MutS proteins detect mismatches? It appears that their *modus operandi* involves testing DNA for weakened Watson–Crick hydrogen bonding and base–base stacking interactions, rather than searching for elusive structural features common to base–base mismatches and insertion/deletion loops. One feature of the DNA that would appear to be important here is its pliability. The protein needs to bend the substrate in order to gain access to the minor groove, and the energy required to do this is most likely lower at mismatch sites than in regular B-form DNA. The relevance of DNA bending to MutS substrate recognition is emphasised by the finding that the human MSH2–MSH6 heterodimer binds DNA modified with cisplatin [8], which is known to bend the helix by about 70°.

What the crystal structures failed to reveal is what role the ATPase activity plays in the mismatch repair process. The composite ATPase domain of MutS is closely related to those found in DNA repair proteins UvrA and Rad50 [9], as well as those in ‘ABC’ family ATPases, such as the cystic fibrosis gene product CFTR, the multi-drug resistance protein Mdr, or the histidine permease HisP [10]. The binding and hydrolysis of ATP is known to bring about major conformational changes in these proteins, and the MutS homologues are no exception. In the absence of a nucleotide cofactor, or in the presence of ADP, MutS and heteroduplex DNA form stable complexes. In contrast, in the presence of ATP, *E. coli* MutS has been proposed to leave the mismatch site [11] and the eukaryotic MSH2–MSH6 complexes behave similarly [12–15].

Because the presence of a mismatch must be signalled to the distal strand interruption, where the exonucleolytic process of mismatch correction actually commences [1], the translocation ability of the mismatch-binding factor is perhaps not too surprising. How it does this is the subject of some controversy, however: it could slide in a hydrolysis-independent mode, where release of the protein from the mismatch could be mediated simply by ATP-induced movement of the thumb tip out of the mismatch site without affecting the finger domains, or it could travel by moving alternate finger domains along the DNA through the binding and hydrolysis of ATP in one or the other subunit. The finding that the *E. coli* MutS–DNA co-crystal has an ADP molecule bound in only one subunit [2] might appear to lend support to the stepwise mode of translation, but this needs to be studied in more detail.

Surprisingly, the authors of both structure papers [2,3] speculate that the MutS protein remains bound at the mismatch site, marking thus the position where the repair reaction has to terminate. Can MutS stay and travel at the same time? Unlikely as it may seem, there may be a way. In the presence of ATP, the protein has been shown to generate loops on long heteroduplex DNA molecules [11]. This result is hard to explain based on the crystal structure, unless the functional unit of MutS were not a dimer, but a tetramer. In such a case, one MutS dimer could bind at the mismatch site and remain there, while the other could associate with homoduplex DNA and, with the aid of its ATP-driven motor, travel along the DNA until it has reached the strand discrimination signal.

It is noteworthy that MutS readily forms tetramers; indeed, the carboxyl terminus of the protein, which is thought to be largely responsible for this association, was deleted for the structural studies because otherwise tetramerisation interfered with crystallisation. As this domain is dispensable for mismatch repair *in vivo*, its role in the process was dismissed. But the tetramer might still form, thanks to interactions with other mismatch repair proteins such as MutL [11]. Studies of MutS–MutL interactions, currently ongoing in several laboratories, should throw some light onto this issue.

The discovery of the 'praying hands' structure of the MutS proteins of *E. coli* and *T. aquaticus* is an important breakthrough in our understanding of the complex molecular transactions that take place during mismatch correction. But this is but the first step on a stairway to heaven…

Acknowledgements

I would like to thank Peggy Hsieh, Titia Sixma and Wei Yang for making their data available to me prior to publication, and apologise to those colleagues, whose contributions are not cited here due to the length constraints of this article.

References

1. Modrich P: **Mechanisms and biological effects of mismatch repair.** *Annu Rev Genet* 1991, **25:**229-253.
2. Lammers MH, Perrakis A, Enzlin J, Winterwerp HHK, de Wind N, Sixma TK: **The crystal structure of DNA mismatch repair protein MutS binding to a GT mismatch.** *Nature* 2000, **407:**711-717.
3. Obmolova G, Ban C, Hsieh P, Yang W: **Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA.** *Nature* 2000, **407:**703-710.
4. Malkov VA, Biswas I, Camerini-Otero RD, Hsieh P: **Photocross-linking of the NH2-terminal region of Taq MutS protein to the major groove of a heteroduplex DNA.** *J Biol Chem* 1997, **272:**23811-23817.
5. Bowers J, Sokolsky T, Quach T, Alani E: **A mutation in the MSH6 subunit of the *Saccharomyces cerevisiae* MSH2–MSH6 complex disrupts mismatch recognition.** *J Biol Chem* 1999, **274:**16115-16125.
6. Dufner P, Marra G, Räschle M, Jiricny J: **Mismatch recognition and DNA-dependent stimulation of the ATPase activity of hMutSα are abolished by a F432A mutation in the hMSH6 subunit.** *J Biol Chem* 2000, in press.
7. Kennard O: **Structural studies of DNA fragments: the G.T wobble base pair in A, B and Z DNA; the G.A base pair in B-DNA.** *J Biomol Struct Dyn* 1985, **3:**205-226.
8. Duckett DR, Drummond JT, Murchie AI, Reardon JT, Sancar A, Lilley DM, Modrich P: **Human MutSα recognizes damaged DNA base pairs containing O_6-methylguanine, O_4-methylthymine, or the cisplatin-d(GpG) adduct.** *Proc Natl Acad Sci USA* 1996, **93:**6443-6447.
9. Gorbalenya AE, Koonin EV: **Superfamily of UvrA-related NTP-binding proteins. Implications for rational classification of recombination/repair systems.** *J Mol Biol* 1990, **213:**583-591.
10. Hung LW, Wang IX, Nikaido K, Liu PQ, Ames GF, Kim SH: **Crystal structure of the ATP-binding subunit of an ABC transporter.** *Nature* 1998, **396:**703-707.
11. Allen DJ, Makhov A, Grilley M, Taylor J, Thresher R, Modrich P, Griffith JD: **MutS mediates heteroduplex loop formation by a translocation mechanism.** *EMBO J* 1997, **16:**4467-4476.
12. Studamire B, Quach T, Alani E: ***Saccharomyces cerevisiae* Msh2p and Msh6p ATPase activities are both required during mismatch repair.** *Mol Cell Biol* 1998, **18:**7590-7601.
13. Blackwell LJ, Martik D, Bjornson KP, Bjornson ES, Modrich P: **Nucleotide-promoted release of hMutS-α from heteroduplex DNA is consistent with an ATP-dependent translocation mechanism.** *J Biol Chem* 1998, **273:**32055-32062.
14. Gradia S, Subramanian D, Wilson T, Acharya S, Makhov A, Griffith J, Fishel R.: **hMSH2-hMSH6 forms a hydrolysis-independent sliding clamp on mismatched DNA.** *Mol Cell* 1999, **3:**255-261.
15. Iaccarino I, Marra G, Dufner P, Jiricny J: **Mutation of the magnesium binding site of hMSH6 disables the hMutS-α sliding clamp from translocating along DNA.** *J Biol Chem* 2000, **275:**2080-2086.